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Briscoe, Kurt G. Norris McLaughlin & Marcus, PA 875 Third Avenue, 8th Floor New York, NY 10022			EXAMINER SINGH, ANOOP KUMAR	
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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No. 10/685,837	Applicant(s) SEIBLER ET AL.	
	Examiner ANOOP SINGH	Art Unit 1632	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

- 1) ☒ Responsive to communication(s) filed on 14 September 2009.
- 2a) ☐ This action is **FINAL**. 2b) ☒ This action is non-final.
- 3) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

- 4) ☒ Claim(s) 1,5,6,9-12,15-17,20-24,26,27 and 30-38 is/are pending in the application.
- 4a) Of the above claim(s) _____ is/are withdrawn from consideration.
- 5) ☐ Claim(s) _____ is/are allowed.
- 6) ☒ Claim(s) 1, 5-6, 9-12, 15-17, 20-24, 26-27, 30-37 and 38 is/are rejected.
- 7) ☐ Claim(s) _____ is/are objected to.
- 8) ☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

- 9) ☐ The specification is objected to by the Examiner.
- 10) ☐ The drawing(s) filed on _____ is/are: a) ☐ accepted or b) ☐ objected to by the Examiner.
Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).
Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).
- 11) ☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

- 12) ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
- a) ☐ All b) ☐ Some * c) ☐ None of:
1. ☐ Certified copies of the priority documents have been received.
 2. ☐ Certified copies of the priority documents have been received in Application No. _____.
 3. ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

- | | |
|---|---|
| 1) <input checked="" type="checkbox"/> Notice of References Cited (PTO-892) | 4) <input type="checkbox"/> Interview Summary (PTO-413) |
| 2) <input type="checkbox"/> Notice of Draftperson's Patent Drawing Review (PTO-948) | Paper No(s)/Mail Date. _____ |
| 3) <input type="checkbox"/> Information Disclosure Statement(s) (PTO/SB/08) | 5) <input type="checkbox"/> Notice of Informal Patent Application |
| Paper No(s)/Mail Date _____ | 6) <input type="checkbox"/> Other: _____ |

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DETAILED ACTION

A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 09/14/2009 has been entered.

Applicant's amendments to the claims filed September 14, 2009 have been entered. Claims 2-4, 7-8, 13-14, 18-19, 25, 28 and 29 have been canceled, while claims 1, 9, 22, 26-27, 30 and 31 have been amended. Claims 1, 5-6, 9-12, 15-17, 20-24, 26-27, 30-37 and 38 are pending in this application.

Election/Restrictions

Applicant's election with traverse of the invention of group IV (27) filed October 24, 2005 was acknowledged. Applicant's argument of examining method for gene knock down in a vertebrate (group 1) with elected group were found persuasive, therefore invention of group I and IV directed to vertebrate and method of gene knock down in a vertebrate were rejoined for the examination purposes. Applicants have also elected SEQ ID NO: 23 as species for claims 31-38. The restriction was deemed proper and therefore made FINAL.

Claims 1, 5-6, 9-12, 15-17, 20-24, 26-27, 30-38 are under current examination.

Priority

It is noted that instant application claims benefit from application number 60/485,969 07/10/2003 that claims benefit of 60/467,814 filed on 05/02/2003, which claims benefit from 60/420,476 filed on 10/22/2002. Upon review of the disclosure of the prior-filed application, '969, '814 and '476 fails to provide descriptive support for instant claims 31-35, 37-38 generic for elected species of SEQ ID NO: 23. There is not adequate support or enablement for claims 31-35, 37-38 in the manner provided by the first paragraph of 35 U.S.C. 112 in any of these

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applications. In case, if applicants have evidence to support otherwise, applicants are invited to indicate page and line number for the written support as recited in claims 31-35, 37-38 generic for SEQ ID NO: 23 of the instant application. Therefore, the effective filing date for instant claims 31-35, 37-38 that is generic for SEQ ID NO: 23 is 10/15/2003, while the subject matter of claims 1, 5-6, 9-12, 15-17, 20-24, 26-27, 30 was described in the application no. 60/420476.

Withdrawn-Claim Objections

Applicants' amendments to claim 1 obviates the basis of the objection. It is noted that applicants have amended claim to recite "mouse" commensurate with the scope of preamble and rest of the claims.

New-Claim Objections

Claim 26 is objected to because of the following informalities: In the instant case, claim 26 recites "the nonhuman vertebrate" in line 3, which is not commensurate with the scope of preamble and rest of the claims. Recitation of "mouse" in place of "nonhuman vertebrate" would obviate the basis of objection. Appropriate correction is required.

Claim Rejections- 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 27 and 38 remain rejected under rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a mouse having stably integrated an expression vector comprising a shRNA construct under control of a promoter selected from a group consisting of snRNA promoter, a RNase P RNA promoter, a trna promoter, a 7SL RNA promoter, and a 5 S Rrna promoter; at a polymerase II dependent locus of the mouse genome by homologous recombination, wherein expression of said shRNA results in reduced expression of the gene targeted by said shRNA in said mouse, does not reasonably provide enablement for a

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mouse without any phenotype. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to use/make the invention commensurate in scope with these claims. Applicants' amendments and arguments filed 09/14/2009 have been fully considered but are not fully persuasive.

Claims 1, 5-6, 9-12, 15-17, 20-24, 26-27 and 37 and 38 were rejected under 35 U.S.C. 112, first paragraph, because the specification fails to provide an enablement for the full scope of the claimed invention. Applicants' cancellation of claims 8, 18 and 19 renders their rejections moot. Applicants' arguments that instant specification teach persons skilled in the art a method to stably integrate the construct into the genome of the mouse, and to test and confirm whether a reduction in the activity of the target gene has been obtained is persuasive. Examiner would agree that it would be routine experimentation to stably integrate the construct comprising shRNA in targeted genomic loci. Therefore, rejection of claims 1, 5-6, 9-12, 15-17, 20-24, 26 and 37 pertaining to method of gene knock down are hereby withdrawn and thereby obviates the grounds for rejection. Applicants' arguments with respect to the withdrawn rejections are thereby rendered moot. However, it was also indicated that the disclosure is not enabled for mouse whose genome comprises a construct comprising shRNA or a construct comprising SEQ ID NO: 23 (ras-gap) without any specific phenotype to make and use the invention. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims. The rejection set forth on pp. 4-11 of the previous office action dated August 20, 2007 is maintained for claims 27 and 38 for the reasons of record.

The claims encompass a mouse that lacks a phenotype. The specification teaches that the purpose of the instant invention is to provide knock down animal with the use of shRNA expression vectors. The guidance provided in the specification is limited to a method to gene suppression of Renilla (Rluc) and luciferase. The specification teaches a method of shRNA within the vector of the invention comprises at least one DNA segment A-B-C wherein A is a 15 to 35, preferably 19 to 29 bp DNA sequence being at least 95%, preferably 100% complementary to the gene to be knocked down (e.g. firefly luciferase, p53, etc.); B is a spacer DNA sequence having 5 to 9 bp forming the loop of the expressed RNA hair pin molecule, and C is a 15 to 35, preferably 19 to 29 bp DNA sequence being at least 85% complementary to the

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sequence A (see para 40-44 of the specification). The specification teaches the expression of the shRNA transgene encoding firefly luciferase by the transgenic mouse efficiently represses firefly luciferase activity in most organs (p. 19, Example 4, last par, lines 7-10). Therefore, the specification provides specific guidance to a transgenic mouse comprising a phenotype.

However, the specification fails to provide specific guidance to teach the use of a transgenic rodent with no phenotype as claimed. Therefore, the specification does not provide an enabled use for a transgenic rodent with no phenotype. The state of the art at the time of filing uses the unpredictability of obtaining transgenic animals with a specific phenotype as summarized by the references of Keri et al., (Proc Natl Acad Sci U S A. 2000; 97(1):383-7) show that elevated levels of lutenizing hormone in transgenic can result in different reproductive system abnormalities including ovarian tumors. Similarly, Carmell et al failed to produce any distinct phenotype, while shRNA, constructs directed against seven known targets were introduced via standard transgenesis in mouse (Carmell MA Nat Struct Biol. 2003; 10(2): 91-92, art of record and Holschneider et al. (Int J Devl Neuroscience, 2000, 18: 615-618). This is particularly critical since it is widely known that a single gene is often essential in a number of different physiological processes. Hence, deletion of changes in an individual gene may prove so drastic or so widespread as to create an amalgam of phenotypes whose interpretation becomes confounded by the interaction of various new physiologic changes (pp 615). It was indicated that claims 27 and 38 do not require any specific phenotype of resulting transgenic mouse and one of skill in art would not know how to use a mouse whose genome comprises a construct comprising SEQ ID NO: 23 or any other construct comprising shRNA. Given that the resulting phenotype of a knockdown mouse was considered unpredictable and it was confounded by multiple compensatory pathways. The specification does not teach any transgenic mouse comprising any knockdown of any level would result in expected phenotype. An Artisan of skill would need to perform further research upon the mouse obtained by the process disclosed in the instant application in order to determine the correlation between the transgene knockdown and the observed phenotypes or effect. In absence of any specific teaching or arguments, an artisan of skill would have to perform undue experimentation to make new invention in the field to make use of the invention.

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While applicants' argument that that claimed method is exactly tailored to the enablement is persuasive for the method claims, applicants should further note that the claims were also rejected on the grounds that the inventions encompasses a transgenic mouse with no phenotype and the specification does not provide an enabled use for a mouse that lacks a phenotype. Applicant did not specifically address this issue of enablement with respect to product claims; thus the rejection pertaining to this issue is maintained for the reasons of record.

Withdrawn-Claim Rejections- 35 USC § 103

Claims 31-35, 37-38 were rejected under 35 U.S.C. 103(a) as being unpatentable over Beach et al. (US. 2003/0084471, dated 5/1/2003, effective date 1/22/2002) or McCaffrey et al., (Nature, 2002 Vol. 418, 38-39, art of record); Bronson et al (Proc Natl Acad Sci U S A 1996; 93:9067-9072, art of record), Soriano et al (US patent 6,461,864, , 2002, art of record) and Kunath et al., (Nature Biotechnology, 21: 559-561, 2003, IDS). In view of Applicants' arguments and upon further consideration in view of newly applied reference of Lowe et al, the previous rejection is hereby withdrawn and thereby rendered moot.

New-Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negated by the manner in which the invention was made.

Claims 31-35, 37-38 are rejected under 35 U.S.C. 103(a) as being unpatentable over Lowe et al (US 2008/0226553, dated 9/18/2008; effective filing date: 9/27/2003), Soriano et al (US patent 6,461,864, October 8, 2002, art of record) and Kunath et al., (Nature Biotechnology, 21: 559-561, 2003, IDS).

It should be noted that claim 1, 5-6, 9-10, 15-16, 20-24, 26-27 and 30 are not included in the rejection because the claimed subject matter is not disclosed in provisional application no

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60/414,605, dated 9/27/2002. Further, the effective filing date of claims 31-35, 37-38 included in the rejection is 10/15/2003 for the reasons discussed above (see priority section).

Lowe et al teach an expression vector encoding a firefly luciferase shRNA construct flanked by two targeting sequences that target integration of the expression vector to the polymerase II dependent, *hprt* gene locus of a mouse genome. Upon recombination and integration of the expression vector into the *hprt* gene locus, the luciferase shRNA construct is operably linked to the ubiquitous mouse *hprt* promoter (Figure 23). It is also disclosed that the shRNA construct is under control of a ubiquitous promoter as claimed that includes RNA polymerase III U6 snRNA promoter, H1 RNA promoter, tRNA promoter or 7SL RNA promoter (See para. 67-68). Lowe discloses that this expression vector is intended for introduction into mouse embryonic stem (ES) cells (page 17, para, 0172, line 1 to para 0173, line 9). Thus, Lowe clearly discloses all of the limitations of expression vector as claimed. With respect to a method gene knock down, Lowe et al teach providing expression construct comprising shRNA, introducing the luciferase shRNA expression vector, discussed above, into cultured mouse ES cells comprising and expressing a firefly luciferase gene. Lowe further discloses that said introduction of the luciferase shRNA expression vector results in high levels of site specific integration of the expression vector into the *hprt* gene of the mouse ES cells (page 17, para. 0173, line 1-9). Lowe discloses that expression of the luciferase shRNA expression construct by the mouse ES cells effectively suppressed firefly luciferase activity in the ES cells (page 17, para. 0174], lines 1-10). With regard to claim 38, Lowe discloses that the shRNA expression construct and ES cells comprising the shRNA expression construct, as discussed above, are part of a system for creating genetically defined RNAi "epi-alleles" in mice using Cre-mediated recombination to stably integrate a single RNAi expression cassette into a single locus in the mouse genome. Lowe discloses that this technique will minimize clonal variation due to random integration events. Lowe discloses that the system was developed to mediate the integration of a single shRNA expression cassette into mouse ES cells (page 17, para 0172, lines 1-20). While Lowe et al teach stable integrating the shRNA construct in *hprt* locus but differ from claimed invention by not disclosing integration of construct into the *rosa 26* gene locus.

However, such was known in prior art. For instance, Soriano et al teach methods and vector constructs for the production of genetically engineered non-human animals including

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mouse, which ubiquitously express a heterologous DNA segment in Rosa 26 locus (abstract and claim 1, col. 9, lines 55-6539). It is noted that Soriano describes targeting region as a portion of a targeting construct which becomes integrated into an endogenous chromosomal location following homologous recombination between a homology clamp and an endogenous gene locus, such as a ROSA26, ROSA5, ROSA23, ROSA11, G3BP (BT5), or EphA2 gene locus sequence (column 3, lines 51-54). Soriano taught a method of targeting region that is flanked on each side by a homology clamp, such that a double-crossover recombination between each of the homology clamps and their corresponding endogenous gene sequences result in replacement of the portion of the endogenous gene locus by the targeting region. However Soriano et al differed from instant method by not disclosing the target sequence being SEQ ID NO: 23 under the control of RNA pol III promoter.

Kunath et al cure the deficiency by teaching a construct comprising DNA encoding the human H1 RNA pol III promoter and a RasGAP shRNA sequence (SEQ ID NO: 23, 100% sequence homology) (see page 561, col. 1, para. 3). Regarding claims 37 and 38, Kunath et al teach a method of gene knockdown by providing the expression vector comprising SEQ ID NO: 23 that are integrated in the genome of ES cells that resulted in inhibition of RasGAP protein (see figure 1 and 2). It is noted that the shRNA disclosed by Kunath et al comprises at least one DNA segment A-B-C wherein A is a 15 to 29 bp DNA sequence with at least 100% complementarily to the gene to be knocked down; B is a spacer DNA sequence having 5 to 9 bp forming the loop of the expressed RNA hairpin molecule, and C is a 19 to 329 bp DNA sequence and further comprises a poly A sequence meeting the limitation of claims (see figure 1A).

It would have been obvious for one of ordinary skill in the art at the time of invention to modify the method of gene knock down disclosed by Lowe et al to include shRNA expression cassettes that are flanked by homology regions for the rosa 26 dependent locus by homologous recombination in ES cells to generate mouse having a single-copy of a transgene inserted at a chosen site in the genome as disclosed by Soriano. The reference of Soriano provided guidance with respect to ubiquitously expressed gene loci to use Rosa 26, rosa5 (see col. 3, lines 49-54). It would have been obvious for one of ordinary skill in the art to further modify the targeting sequence of Lowe by substituting shRNA sequence with another such as one disclosed by Kunath and then flanking by homology regions for the Rosa26 locus to stably integrate

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expression cassette comprising an shRNA under control of ubiquitous pol III promoter into a specific genomic locus such as HPRT/rosa26 as discussed by Lowe and Soriano with reasonable expectation of achieving predictable result to efficiently suppress the transgene expression. It is noted that several polymerase II dependent loci were known at the time of filing of this application and it would have required only routine experimentation to flank expression cassettes comprising shRNA under the control of a promoter with the homology regions of other polymerase II dependent locus (See MPEP2144.04). One who would practiced the invention would have had reasonable expectation of success because Lowe provided guidance with respect to produce a mouse comprising an expression vector comprising a shRNA construct that integrates into a polymerase II dependent locus and results in suppression of expression of the gene targeted by said shRNA, while Kunath provided guidance with respect to specific SEQ ID NO: 23. Thus, it would have only required routine experimentation to modify the expression construct disclosed by Kunath that are flanked by homology regions for the polymerase II dependent locus as disclosed by Lowe and Soriano. One of ordinary skill in the art would have combined the teaching of Lowe et al, Soriano and with Kunath because a method of gene knockdown in a mouse comprising a shRNA construct under control of a RNA polymerase III promoter into a specific polymerase II dependent locus that included hpert, rosa26 would have provided stable and sustained expression of short hairpin resulting in gene knockdown.

Therefore, the claimed invention would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention.

Maintained-Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negated by the manner in which the invention was made.

Claims 1, 5-6, 9-10, 15-16, 20-24, 26-27 and 30 remain rejected under 35 U.S.C. 103(a) as being unpatentable over McCaffrey et al., (Nature, 2002 Vol. 418, 38-39) or Beach et al. (US

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patent Publication no. 2003/0084471, dated 5/1/2003, effective filing date 1/22/2002) and Bronson et al (Proc Natl Acad Sci U S A 1996; 93:9067-9072).

Claim 1 is directed to a method comprising (i) stably integrating by homologous recombination an expression vector comprising shRNA construct under the control of a RNA polymerase III promoter and homologous sequence which integrates through homologous recombination at polymerase II dependent locus. It is emphasized that instant rejection is to the breadth of the claims.

McCaffrey et al teach a method of gene knock down in a transgenic mouse comprising an expression vector comprising shRNA under the control of ubiquitous promoter (see abstract). McCaffrey et al teaches delivering an expression vector comprising small-hairpin RNAs (shRNAs) that is expressed *in vivo* from DNA templates using RNA polymerase III promoters inhibiting the luciferase expression by up to 98% (pp38, Figure 1 C-D and pp39 2nd paragraph). McCaffrey teaches shRNA that comprises at least one DNA segment A-B-C wherein A is a 15 to 29 bp DNA sequence with at least 100% complementarity to the gene to be knocked down; B is a spacer DNA sequence having 5 to 9 bp forming the loop of the expressed RNA hairpin molecule, and C is a 19 to 329 bp DNA sequence and further comprises a poly A sequence meeting the limitation of claims 20-24 (see the supplementary information). Although, McCaffrey et al taught a method of gene knockdown in a mouse, but differed from claimed invention by not disclosing stably integrating the expression construct in a polymerase II dependent locus.

Beach et al disclose that the double-stranded structure may be formed by a single self-complementary RNA strand or two complementary RNA strands. RNA duplex formation may be initiated either inside or outside the cell. Inhibition is sequence-specific in that nucleotide sequences corresponding to the duplex region of the RNA are targeted for genetic inhibition (pp4, paragraph 52). Beach et al teach the length of the dsRNA is at least 20, 21 or 22 nucleotides in length, e.g., corresponding in size to RNA products produced by Dicer-dependent cleavage. In certain embodiments, the dsRNA construct is at least 25, 50, 100, 200, 300 or 400 bases (pp13, paragraph 16). Beach et al disclose that the dsRNA construct may be synthesized either *in vivo* or *in vitro*. RNA can be derived from an expression construct (pp 13,14; paragraph 168). The invention also discloses strategy for stable expression of dsRNA in cultured mammalian cells (Figure 27, paragraph 78). Beach et al disclose generating several types of short dsRNAs corresponding to the coding region of firefly or Renilla luciferase (pp22; paragraph 246). Beach et al demonstrates that short hairpins encoded on a plasmid are effective in suppressing luciferase gene expression (Figure 42) *in vivo*. DNA oligonucleotide encoding 29 nucleotide hairpins corresponding to firefly luciferase were inserted into a vector containing the U6 promoter. Beach further discloses that one of skill can choose from amongst a range of vectors to either transiently or stably express a short hairpin. Beach et al also disclose non-limiting examples of vectors and strategies to stably express short dsRNAs using U6 and H1 promoters (pp23; paragraph 252; Figures. 43-45). It is noted that Beach et al also disclose that promoters/enhancers that may be used to control the expression of the targeted gene *in vivo* may include cytomegalovirus (CMV) promoter (see para. 147). Beach et al teach and claim a non-

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human transgenic vertebrate selected from a list consisting from mouse (see page 12, para. 154) having germline and/or somatic cells comprising a transgene encoding a dsRNA construct (pp 26, claim 28 and pp 2 paragraph 52) that includes rodent (pp12, paragraph 154). Beach et al also demonstrates that a short hairpin is highly effective in specifically suppressing gene expression of firefly or Renilla luciferase (Example 6). However, Beach et al do not explicitly teach how an expression vector integrates through homologous recombination at polymerase II dependent locus.

Prior to instant invention, Bronson describes transgenic mice made by pro nuclear injection of DNA as an effective method of achieving expression of exogenous DNA sequences for many purposes, including over expression, mutant analysis, promoter analysis (see page 9067, column 1, para 1). Bronson also describes problems associated with DNA incorporated into the mouse germ line using this method includes random integration and unpredictable copy numbers. This random integration often also presents profound effect on expression of the transgene resulting in altered phenotype of the mouse (see page 9067, col.1, para. 1). It is noted that Bronson provided advantages of targeting a single copy of a transgenic sequence to a chosen location in the genome such as HPRT over random integration of construct. He discloses many advantages of targeting at specific locus including the ability to control copy number, the ability to insert the transgene into regions of chromatin compatible with a desired developmental and tissue-specific expression. It is noted that Bronson et al emphasize that targeted transgenes provide a more efficient and informative means of securing and comparing the expression of various transgenic sequences than is available with current transgenic procedures. Bronson also taught homologous recombination in murine ES cells to generate mice having a single-copy of a transgene inserted at a chosen site in the genome (see page figure 2 and page 9068, column 2, para 3). Specifically, Bronson et al disclose a method wherein a single copy murine bcl-2 cDNA driven by either a chicken beta-actin promoter or a human beta-actin promoter has been inserted immediately 5' to the HPRT locus by a directly selectable homologous recombination event (see the abstract and figure 2). However, Bronson et al do not teach expressing shRNA in a specific locus.

It would have been obvious for one of ordinary skill in the art at the time of invention to modify the method of gene knock down disclosed by McCaffrey or Beach to include the shRNA expression cassettes that are flanked by homology regions for the polymerase II dependent locus (HPRT) as disclosed by Bronson to stably integrate by homologous recombination in ES cells to generate nonhuman vertebrate having a single-copy of a transgene inserted at a chosen site in the genome. Bronson provided guidance by emphasizing that the use of a chosen site for a single copy of a transgene avoids many of the problems associated with randomly inserted transgenes (see page 9072, col. 1, last paragraph). It would have been prima facie obvious for one of ordinary skill in the art to make transgenic nonhuman animal that comprises stably integrated expression vector comprising an shRNA into a specific locus such as HPRT by homologous recombination as discussed by Bronson in order to more efficiently suppress the transgene expression for sustained period. One who would practiced the invention would have had reasonable expectation of success because McCaffrey/Beach et al had already described a method for gene knockdown in a mice by transiently as well as stably expressing shRNA construct and it would have only required routine experimentation to modify the expression construct that are flanked by homology regions for the polymerase II dependent locus as

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disclosed by Bronson. One of ordinary skill in the art would have been studied Bronson to combine the teaching of Beach/ McCaffrey because a method of gene knockdown in a mouse comprising a shRNA construct under control of a ubiquitous promoter into a specific polymerase II dependent locus would have provided stable and sustained expression of short hairpin resulting in gene knockdown.

Therefore, the claimed invention would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention.

Claims 1, 5, remain rejected under 35 U.S.C. 103(a) and claims 31-34, 36-38 are also rejected under 35 U.S.C. 103(a) as being unpatentable over Beach et al. (US patent Publication no. 2003/0084471, dated 5/1/2003, effective filing date 1/22/2002) or McCaffrey et al., (Nature, 2002 Vol. 418, 38-39, art of record); Bronson et al (Proc Natl Acad Sci U S A 1996; 93:9067–9072, art of record) as applied to claims 1, 5-6, 8-10, 15-16, 18, 20-24, 26-27 above, and further in view of Soriano et al (US patent 6,461,864, October 8, 2002, art of record). Claims 31-34, 36-38 are included in the rejection to the extent claims read on the limitation of claim 36 (shRluc and shFluc).

The teaching of Beach et al or McCaffrey and Bronson et al have been discussed above and relied in same manner here. Although combination of Beach /McCaffrey and Bronson taught a method of stably integrate by homologous recombination an shRNA construct under the control of a promoter in polymerase II dependent locus (HPRT) but differed from claimed invention by not disclosing stably integrating into other polymerase II dependent locus such as Rosa26.

Soriano et al teach methods and vector constructs for the production of genetically engineered non-human animals, which ubiquitously express a heterologous DNA segment in Rosa 26 locus (abstract and claim 1). It is noted that Soriano describes targeting region as a portion of a targeting construct which becomes integrated into an endogenous chromosomal location following homologous recombination between a homology clamp and an endogenous gene locus, such as a ROSA26, ROSA5, ROSA23, ROSA11, G3BP (BT5), or EphA2 gene locus sequence (column 3, lines 51-54). Soriano taught a method of targeting region that is flanked on each side by a homology clamp, such that a double-crossover recombination between each of the homology clamps and their corresponding endogenous gene sequences result in replacement of the portion of the endogenous gene locus by the targeting region. However Soriano et al differed from instant method by not disclosing using shRNA construct in rosa26 locus.

It would have been obvious for one of ordinary skill in the art at the time of invention to modify the method of gene knock down disclosed by McCaffrey/Beach to include shRNA expression cassettes that are flanked by homology regions for the polymerase II dependent locus (rosa26) by homologous recombination in ES cells to generate nonhuman vertebrate having a single-copy of a transgene inserted at a chosen site in the genome. Bronson provided guidance by emphasizing that the use of a chosen site for a single copy of a transgene avoids many of the problems associated with randomly inserted transgenes (see supra and page 9072, col. 1, last

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paragraph). The reference of Soriano provided guidance with respect to ubiquitously expressed gene loci for use include Rosa 26, rosa5 and others (see col. 3, lines 49-54). It would have been obvious for one of ordinary skill in the art to try a method of gene knock down in nonhuman vertebrate by modifying the shRNA expression cassettes under the control of the CMV/H1 or U6 promoter as disclosed by McCaffrey/Beach and then flanking by homology regions for the Rosa26 locus to stably integrate expression cassette comprising an shRNA under control of ubiquitous promoter into a specific locus such as HPRT/rosa26 as discussed by Bronson with reasonable expectation of achieving predictable result to more efficiently suppress the transgene expression. It is noted that several polymerase II dependent loci were known at the time of filing of this application and it would have required only routine experimentation to flank expression cassettes comprising shRNA under the control of a promoter with the homology regions of other polymerase II dependent locus (See MPEP2144.04). One who would practiced the invention would have had reasonable expectation of success because McCaffrey/Beach had already described a method for gene knockdown in a mice by random integration of the construct and it was routine to use express transgene in a chosen site to avoid many of the problems associated with randomly inserted transgenes as evidenced from the teaching of Bronson. Thus, it would have only required routine experimentation to modify the expression construct that are flanked by homology regions for the polymerase II dependent locus as disclosed by Bronson. One of ordinary skill in the art would have been studied Bronson to combine the teaching of Beach/McCaffrey and Soriano because a method of gene knockdown in a mouse comprising a shRNA construct under control of a ubiquitous promoter into a specific polymerase II dependent locus that included hppt, rosa26 or any other endogenous loci would have provided stable and sustained expression of short hairpin resulting in gene knockdown.

Therefore, the claimed invention would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention.

Claims 11-12, 17 are rejected under 35 U.S.C. 103(a) as being unpatentable over Beach et al. (US patent Publication no. 2003/0084471, dated 5/1/2003, effective filing date 1/22/2002, art of record) or McCaffrey et al., (Nature, 2002 Vol. 418, 38-39, art of record); Bronson et al (Proc Natl Acad Sci U S A 1996; 93:9067-9072, art of record) and Soriano et al (US patent 6,461,864, October 8, 2002) as applied to claims 1, 5-6, 9-10, 14-16, 20-24, 26-27 and 30 above, and further in view of Ohkawa et al (Hum Gene Ther. 2000; 11 (4): 577-85; IDS).

The combined teachings of or McCaffrey/Beach, Bronson and Soriano have been discussed above and are relied upon in same manner. However, none of the reference explicitly teaches an inducible system.

Ohkawa et al teach several constructs composed of the human U6 snRNA promoter and sequences derived from the gene for the tetracycline operator of a prokaryotic tetracycline resistance transposon (abstract). Ohkawa also disclose that expression of the promoter of the human gene for U6 snRNA that contains tet O sequences between the PSE (Figure. 1 and 2) and a TATA box could be efficiently repressed in cells with the Tet repressor and that this

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repression can be reversed by tetracycline. Ohkawa et al used this expression system to control the function of an antisense RNA for a fusion gene composed of genes for epidermal growth factor receptor (EGFR) and green fluorescent protein (GFP) and expression of this chimeric gene could be efficiently and rapidly inhibited by tetracycline. However Ohkawa et al do not teach a method to gene knockdown in a nonhuman vertebrate.

It would have been obvious for one of ordinary skill in the art at the time of invention to modify the construct and method disclosed by McCaffrey/Beach to include inducible promoters for shRNA construct wherein operator sequence consist tet as disclosed by Ohkawa. One of ordinary skill in the art would be further motivated to include this construct in a specific locus by homologous recombination in ES cells to generate nonhuman vertebrate having a single-copy of a transgene inserted at a chosen site in the genome wherein transgene could be regulated by tetracycline. Ohkawa provided the provided motivation by showing that tet based system could control the expression of transgene, while Bronson emphasized the use of a chosen site for a single copy of a transgene avoids many of the problems associated with randomly inserted transgenes (see page 9072, col. 1, last paragraph). Furthermore, Bronson and Soriano provided guidance with respect to different endogenous loci including Rosa 26 locus. The person of ordinary skill in the art would have been studied Bronson to make transgenic nonhuman animal comprising stably integrated expression vector comprising an shRNA under the control of ubiquitous promoter into a specific locus such as ROSA26 or HPRT.

One who would practiced the invention would have had reasonable expectation of success because McCaffrey/Beach had already described a method for gene knockdown in a mice by random integration. It would have only required routine experimentation to combine the teaching of McCaffrey/Beach, Bronson, Ohkawa and Soriano because a method of gene knockdown in a mouse comprising a shRNA construct under control of a tet based inducible promoter into a specific ROSA26/HPRT locus would have provided stable and sustained regulated inhibition of transgene.

Therefore, the claimed invention would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention.

Response to arguments

Applicants' arguments filed September 14, 2009 have been fully considered but are not persuasive. Applicants argue that base claim has been amended to require that the shRNA construct be under the control of a ubiquitous polymerase III dependent promoter and be integrated into a polymerase II dependent locus of the mouse genome. As is apparent from the instant examples, Applicants have found that a single copy shRNA construct under the control of a polymerase III dependent promoter can mediate ubiquitous RNA interference in a living organism when integrated into a RNA polymerase II dependent locus. And, as discussed in the instant specification in the second paragraph on page 2 and the second paragraph on page 3, this finding was by no means obvious to persons having ordinary skill in the art. The combinations of

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McCaffrey or Beach and Bronson alone or further in view of Soriano, Ohkawa and/or Kunath do not teach or suggest to persons having ordinary skill in the art a single copy shRNA construct under the control of a polymerase III dependent promoter can mediate ubiquitous RNA interference in a living organism when integrated into a RNA polymerase II dependent locus. McCaffrey, Beach and Kunath teach a method of gene knockdown in a mouse by administering a shRNA expression vector, but fail to teach the strategy of targeted integration of an shRNA construct under the control of a polymerase III dependent promoter into a RNA polymerase II dependent locus to achieve ubiquitous RNA interference in a living organism (see page 15 and 16 of the arguments).

In response to applicant's arguments against the references individually, one cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references. See *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981); *In re Merck & Co.*, 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986). Applicants have further engaged in selective reading of the teachings of Bronson and Soriano et al. to formulate the grounds for teaching away. It should be noted that the prior art recognized that the local chromosomal environment can affect expression of the integrated transgene and this can result in unpredictable transgene expression. Bronson et al provide motivation to integrate construct at a specific genomic loci by homologous recombination in order to overcome the problem of random transgene integration. This method allows the introduction of single copy transgene into the X-linked hprt locus (see Bronson et al entire article). The art teaches introducing single copy transgene to 5' of the hprt locus by homologous recombination in ES cells. Further the teaching of Bronson also suggests that the hprt locus is a particularly suitable site for the integration of transgenes because it exists as an X-linked gene present as a single copy in male ES cells. Moreover, the hprt gene is ubiquitously expressed and so provides a favorable chromatin environment for transgene expression. Bronson et al also show that the level of expression of transgenes inserted into the hprt locus is directed solely by exogenous transcriptional regulatory elements (emphasis added) (see page 9071, col. 2, para. 1). Thus, Bronson et al. cure the deficiency in McCaffrey/ Beach et al of introducing an expression vector comprising small-hairpin RNAs (shRNAs) that is randomly integrated and expressed *in vivo* from DNA templates using RNA polymerase III promoters inhibiting the luciferase expression by up to 98% (supra).

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To the extent that Bronson et al. describe the single copy integration of a transgene at *hprt* locus by homologous recombination to produce stable expression, the rejection is applicable to the instant case.

Applicants' selective reading of McCaffrey/ Beach et al. ignores the teachings of the reference of Bronson et al. There is no requirement for McCaffrey/ Beach et al. to teach that which is clearly taught by Bronson. A person of skill in the art would be motivated to stably integrate the construct comprising the transgene (shRNA) construct disclosed by McCaffrey/ Beach et al into the *hprt* locus by homologous recombination method disclosed by Bronson, because the method would provides a favorable chromatin environment for transgene expression, with a reasonable expectation of success.

With respect to applicants' argument that Bronson et al do not provide motivation to stably integrate construct comprising shRNA under the control of polymerase III promoter in polymerase II dependent locus, applicants should note that it is well established in case law that a reference must be considered not only for what it expressly teaches, but also for what it fairly suggests. *In re Burkel*, 201 USPQ 67 (CCPA 1979). Furthermore, in the determination of obviousness, the state of the art as well as the level of skill of those in the art is important factors to be considered. The teaching of the cited references must be viewed in light of these factors. Furthermore, obviousness does not require absolute predictability of success; for obviousness under 35 U.S.C. § 103, all that is required is a reasonable expectation of success. See *In re O'Farrell*, 7 USPQ2d 1673 (CAFC 1988). In the instant case, the expression vector and transgenic mouse set forth in the independent claims do not distinguish to the expression vector and the resulting mouse disclosed by the combination of cited references. McCaffrey/Beach both teach a method of gene knock down in a mouse by administering an expression vector comprising shRNA under the control of RNA polymerase III dependent promoter that integrates in the mouse genome and achieve greater than 30% reduction in the expression of gene product. The deficiency of McCaffrey/Beach is cured by Bronson who describes problems associated with DNA incorporated into the mouse germ line using this method includes random integration and unpredictable copy numbers. Bronson emphasize that random integration often also presents profound effect on expression of the transgene resulting in altered phenotype of the mouse (see page 9067, col.1, para. 1). It is also disclosed that the level of expression of transgenes inserted

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into the *hprt* locus is directed solely by exogenous transcriptional regulatory elements (emphasis added) (see page 9071, col. 2, para. 1). McCaffrey/Beach et al both teach expression of a shRNA is regulated by RNA polymerase III dependent promoters; such promoters are known to produce efficient silencing (See both McCaffrey/Beach). Bronson et al teach method that uses homologous recombination in embryonic stem (ES) cells to generate mice having a single copy of a transgene integrated into a chosen location in the genome, it would have been *prima facie* obvious for one of ordinary skill in the art to combine the teaching with reasonable expectation of success. Therefore, the claimed invention would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention.

In response to applicant's argument that Soriano did not examine the activity of an exogenous promoter that is stably integrated at *rosa26* (page 18 of the argument), the test for obviousness is not whether the features of a secondary reference may be bodily incorporated into the structure of the primary reference; nor is it that the claimed invention must be expressly suggested in any one or all of the references. Rather, the test is what the combined teachings of the references would have suggested to those of ordinary skill in the art. See *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981).

Applicants should note that if Soriano had disclosed a single copy shRNA construct under the control of a polymerase III dependent promoter mediating ubiquitous RNA interference in a living organism when integrated into a RNA polymerase II dependent locus, this would have been an anticipation rejection. As stated before combination of Beach /McCaffrey and Bronson taught a method of stably integrate by homologous recombination an shRNA construct under the control of a RNA polymerase III promoter in polymerase II dependent locus (HPRT), but differ from claimed invention by not disclosing stably integrating into other polymerase II dependent locus such as *Rosa26*. The deficiency is cured by Soriano et al who provided guidance with respect to ubiquitously expressed gene loci for use include *Rosa 26*, *rosa5* and others (see col. 3, lines 49-54). Soriano et al teach methods and vector constructs for the production of genetically engineered non-human animals, which ubiquitously express a heterologous DNA segment in *Rosa 26* locus (abstract and claim 1). One of ordinary skill in the art would have substituted *hprt* loci disclosed in the method of McCaffrey/ Beach and Bronson with functionally equivalent *rosa26* locus as disclosed by Soriano as a matter of design choice,

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with a reasonable expectation of success, at the time of the instant invention. Said design choice amounting to combining prior art elements according to known methods to yield predictable results.

With respect to applicants' arguments that Soriano did not examine the activity of an exogenous promoter that is stably integrated at genomic locus (see page 18), it should be noted that such is taught by Bronson et al. There is no requirement for Soriano et al. to teach that which is clearly taught by Bronson. A person of skill in the art would be motivated to stably integrate the construct comprising the transgene (shRNA) construct disclosed by McCaffrey/Beach et al into the hprt or rosa26 locus by homologous recombination method as disclosed by Bronson, because the method would provides a favorable chromatin environment for shRNA expression, with a reasonable expectation of success. It should be noted that Bronson et al also show that the level of expression of transgene inserted into the hprt locus is directed solely by exogenous transcriptional regulatory elements (emphasis added) (see page 9071, col. 2, para. 1), while art teaches expression of a shRNA regulated by an RNA polymerase III promoters produce efficient silencing (See both McCaffrey/Beach). Thus, it would have been obvious for one of ordinary skill in the art at the time of invention to modify the method of gene knock down disclosed by McCaffrey/Beach to include shRNA expression cassettes that are flanked by homology regions for the polymerase II dependent locus (rosa26) by homologous recombination in ES cells to generate mouse having a single-copy of a transgene inserted at a chosen site in the genome. Therefore, the claimed invention would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention.

Applicants' arguments filed September 14, 2009 have been fully considered but are not persuasive. Applicant's arguments all rely on the references of McCaffrey/Beach, Bronson and Soriano that has been previously discussed. In absence of any other arguments rejection is maintained for the reasons of record.

Double Patenting

The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible

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harassment by multiple assignees. A nonstatutory obviousness-type double patenting rejection is appropriate where the conflicting claims are not identical, but at least one examined application claim is not patentably distinct from the reference claim(s) because the examined application claim is either anticipated by, or would have been obvious over, the reference claim(s). See, e.g., *In re Berg*, 140 F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); and *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) or 1.321(d) may be used to overcome an actual or provisional rejection based on a nonstatutory double patenting ground provided the conflicting application or patent either is shown to be commonly owned with this application, or claims an invention made as a result of activities undertaken within the scope of a joint research agreement.

Effective January 1, 1994, a registered attorney or agent of record may sign a terminal disclaimer. A terminal disclaimer signed by the assignee must fully comply with 37 CFR 3.73(b).

Claims 1, 5-6, 9-12, 15-17, 20-24, 26-27, 37 and 38 are provisionally rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claim 79-82 copending Application No. 11/571194 (20080313747) in view of Kunath et al., (Nature Biotechnology, 21: 559-561, 2003, IDS).

Although the conflicting claims are not identical, they are not patentably distinct from each other because claim 1 of the instant application is directed to a method for constitutive and/or inducible gene knock down in a mouse, which comprises stably integrating by homologous recombination an expression vector into a polymerase II dependent locus of the genome of the mouse, said expression vector comprising a short hairpin RNA (shRNA) construct under control of a promoter which integrate through homologous recombination at a polymerase II dependent locus of the genome of the non-human vertebrate, wherein the ubiquitous promoter is selected from the group consisting of polymerase III dependent promoters. Subsequent claims limit the polymerase II dependent locus to include from a list elected from the group consisting of Rosa6, Hprt, collagen RNA polymerase. Claims are also directed to limit the vector containing functional sequences selected from the group consisting of splice acceptor sequences, polyadenylation sites and selectable marker sequences. Dependent claims 9-12, 14-17 limit the method of claim 1 to include various promoters. Claims 20-24 limits the shRNA to include a DNA segment A-B-C wherein A is a 15 to 35 bp DNA sequence with at least 95%

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complementarily to the gene to be knocked down; B is a spacer DNA sequence having 5 to 9 bp forming the loop of the expressed RNA hair pin molecule, and C is a 15 to 35 bp DNA sequence with at least 85% complementarily to the sequence A and further comprises a stop and/or polyadenylation sequence. Claim 27 is drawn to a mouse having stably integrated by homologous recombination at a polymerase II dependent locus of the non-human vertebrate an expression vector comprising a short hairpin RNA (shRNA) construct under control of a promoter and homologous sequences which integrate at a polymerase II dependent locus of the genome of the mouse, wherein the ubiquitous promoter is a polymerase III dependent promoters. Claim 37 is directed to a method for gene knock down in a mouse, said method comprising: a) providing an expression vector comprising shRNA sequence set forth in SEQ ID NO: 23 and stably integrating said expression vector into the *rosa26* locus of the genome of embryonic stem cells of said mouse by homologous recombination; and thereby c) achieving an at least 30% reduction in the activity of an expression product of said gene. Claim 38 is directed to a mouse having an expression vector comprising vector of the invention stably integrated into the *rosa26* locus of the genome of cells of said mouse by homologous recombination. In contrast, claims 79-82 of the application no 11/571194 are directed to a transgenic multi-cell organism or a transgenic non-human mammal having a modified ubiquitous locus which comprises transfecting cells comprising a short hairpin RNA construct operatively linked to a promoter or an inactive precursor thereof, which method comprises introducing the expression cassette into the ubiquitous locus of eukaryotic cells by recombinase mediated cassette exchange. Claim 80 is directed to a method for constitutive and/or inducible gene knock down in a multi-cell organism, or in a tissue culture or cells of a cell culture derived from said multi-cell organism, which comprises stably integrating an expression vector comprising a short hairpin RNA construct operatively linked to a promoter, which method comprises introducing the expression cassette into the ubiquitous locus of eukaryotic cells by recombinase mediated cassette exchange (RMCE, which comprises (a) introducing a functional DNA sequence into the *Rosa26* locus of starting eukaryotic cells by homologous recombination with a targeting vector comprising flanking DNA sequences homologous to the ubiquitous locus and an acceptor DNA, which integrates into the genome of the starting cell, the acceptor DNA comprising two mutually incompatible first recombinase recognition sites (RRSs), and (b) effecting RMCE of the

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recombination product of step (a) having RMCE target sites with an exchange vector comprising a donor DNA, which comprises the expression cassette flanked by the same two mutually incompatible first RRSs contained in the acceptor DNA, by utilizing a recombinase which catalyzes recombination into the genome of the living organism. Claim 81 limits the method of claim 79, wherein (i) the expression vector is integrated at the Rosa26 locus of the multi-cell organism, tissue culture or cell culture. Claim 82 limits the method of claim 79, wherein the method for constitutive and/or inducible gene knock down in a vertebrate comprises integrating the expression vector into ES cells of the vertebrate. Kunath et al cure the deficiency by teaching a construct comprising DNA encoding the human H1 RNA pol III promoter and a RasGAP shRNA sequence (SEQ ID NO: 23, 100% sequence homology) (see page 561, col. 1, para. 3). Regarding claims 37 and 38, Kunath et al teach a method of gene knockdown by providing the expression vector comprising SEQ ID NO: 23 that are integrated in the genome of ES cells that resulted in inhibition of RasGAP protein (see figure 1 and 2). It is noted that the shRNA disclosed by Kunath et al comprises at least one DNA segment A-B-C wherein A is a 15 to 29 bp DNA sequence with at least 100% complementarity to the gene to be knocked down; B is a spacer DNA sequence having 5 to 9 bp forming the loop of the expressed RNA hairpin molecule, and C is a 19 to 329 bp DNA sequence and further comprises a poly A sequence meeting the limitation of claims (see figure 1A). Therefore, it would have been obvious to one of skill in the art, at the time the invention was made, to use shRNA sequence disclosed by Kunuth in the method of gene knock down disclosed in '114, with reasonable expectation of achieving the predictable result. Thus, the instant claims are obvious variants of the pending claims when viewed in light of the teachings of Kunuth. It is noted that certain of the instant claims differ only with respect to a narrower scope of promoter and construct, which encompass those specifically claimed in application no 11/571194.

This is a provisional obviousness-type double patenting rejection because the conflicting claims have not in fact been patented.

Claims 1, 5-6, 9-12, 15-17, 20-24, 26-27 and 30 were provisionally rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 14-16, 18-40 of copending Application No. 10/531,347. It is noted that applicants have filed a terminal disclaimer, which was approved on 9/4/2009 to address this rejection in co-pending

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application 10/531,347. Thus, the obviousness-type double patenting rejection of instant claims is hereby withdrawn. Applicants' arguments with respect to the withdrawn rejections are thereby rendered moot.

Conclusion

No Claims allowed.

The prior art made of record and not relied upon is considered pertinent to applicant's disclosure. Gossen et al (Proc Natl Acad Sci U S A. 1992 Jun 15;89(12):5547-51).

Mansour et al., *Proc Natl Acad Sci U S A.* 87(19):7688-92, 1990.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to ANOOP SINGH whose telephone number is (571)272-3306. The examiner can normally be reached on 9:00AM-5:30PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Peter Paras can be reached on (571) 272- 4517. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <http://pair-direct.uspto.gov>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Anoop Singh/
Examiner, Art Unit 1632